Listeria monocytogenes Survival Model Validated in Simulated Uncooked-Fermented Meat Products for Effects of Nitrite and pH

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ABSTRACT

Previous modeling studies in broth cultures demonstrated that acidity and nitrite increased the inactivation rate of *Listeria monocytogenes*. To validate this effect during storage of simulated uncooked-fermented meat products, lean beef was ground with salt, adjusted to pH 4.0–5.1, and treated with nitrite at 0–300 μg/mL. Samples were immediately inoculated with *L. monocytogenes* (10^7 CFU/g) and survivors were enumerated over 21 days storage at 37°C. The time to achieve a four log decline as greatly affected by pH, ranging from 21 days at pH 5.0 to < 1.0 day at pH 4.0. Growth occurred at pH 5.1 after a long lag period. Nitrite additions did not affect survival, suggesting that the effective concentration was the rapidly decreasing residual nitrite level.

Key Words: listeria, meat, fermentation, nitrite, acidity

INTRODUCTION

THERE IS GREAT PUBLIC HEALTH CONCERN about *L. monocytogenes* because it can grow at refrigeration temperatures and low numbers can be infectious to susceptible people. Many traditional and ethnic ready-to-eat meat products are not cooked: these products include nonfermented (Proscuitti, Westphalian hams) and fermented and dried sausages (pepperoni, some salami). *L. monocytogenes* is a bacterial hazard in such products and is frequently present in the environment.

Processors add nitrite to such products for flavor, color fixation, antioxidant effect, and inhibition of *Clostridium botulinum*. Nitrite may also hasten destruction of other bacteria; the experimental data used to develop the ARS predictive models indicate that nitrite decreases survival times of *L. monocytogenes* (Buchanan et al., 1994) as well as slows growth (Buchanan and Phillips, 1990). The ARS model predicts that in a system at pH 4.7, 3.0% NaCl and 37°C the time for *L. monocytogenes* populations to decrease 4 log cycles (T_{4D}) would be 185 hr with no nitrite and 23 hr with 150 ppm nitrite. Note: For the Pathogen Modeling Program, contact Dr. R.L. Buchanan, Microbial Food Safety Research Unit, USDA, ARS, ERRC, 600 E. Mermaid La., Philadelphia, PA 19118, USA. However, because the nitrite level rapidly declines after contacting meat (Nordin, 1969; Dethmers et al., 1975; Tompkin, 1983), it is necessary to determine what nitrite level(s) (initial vs residual) are representative for the model.

Inoculated pack studies usually found reduced numbers of *L. monocytogenes* after fermentation and drying, however, survivors were also detected (Berry et al., 1990; Farber et al. 1993; Johnson et al., 1988; Glass and Doyle, 1989; Karches and Teufel, 1988; Junttila et al., 1989; Sabel et al., 1991). Surveys of fermented meat products confirmed the presence of *L. monocytogenes* in finished products (Bunčić, 1991; Comi et al., 1992; Farber et al., 1988; Trüssel, 1989; Johnson et al., 1990). Currently, comparisons between predictions by models based on broth systems and results of inoculation studies may be questionable because of the dynamic nature of the pH, nitrite levels and water activity during fermentation and drying.

The fermentation process should either kill bacteria or damage them so that any survivors would be destroyed during drying and storage. We determined *L. monocytogenes* survival in meat batters where the pH was controlled and the microorganisms were added immediately after the nitrite, simulating contamination of the product from the plant environment. Our objective was to help establish the validity of the broth model and quantitatively evaluate effects of pH and nitrite under circumstances closer to those of an actual uncooked, fermented meat product.

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MATERIALS & METHODS

Microorganisms

Stock cultures of *L. monocytogenes* strains (Scott A, V7 and HO-VJ) were from the culture collection at the USDA (Philadelphia, PA) and were the same cultures used in developing the broth models (Buchanan and Phillips, 1990; Buchanan et al., 1993, 1994). They were maintained at 4°C on Brain Heart Infusion (BHI) agar (Difco, Detroit, MI). Cultures were grown individually overnight in 25 mL of BHI broth on a rotatory shaker at 37°C. A mixture of equal volumes of the 3 strains was diluted in sterile 0.1% peptone water for inoculation.

Meat samples

Beef round meat, purchased in a local retail store, was chopped in a food processor (Cuisinart, CFP-9). Sodium chloride was added to give a final concentration of 3.0% (w/w) (3.2% brine). The pH was adjusted during chopping by adding a 50% (v/v) lactic acid solution. The relationship between added lactic acid and pH had been previously determined by intermittently adding acid to samples of meat as they were chopped in the food processor. pH was measured by a combination electrode with direct readings. The acidified meat samples (50 or 100g) were placed in Stomacher bags (Seward Medical UAC, London), frozen and irradiated with 3 kG from a ^{137}Cs source. Samples were kept frozen for ≥ 24 h before use. On the day of inoculation, samples were thawed and pH determined. Filter sterilized sodium nitrite was added to give initial concentrations of 0, 150 or 300 ppm. Sterile water was added where necessary to compensate for lactic acid and nitrite additions. Samples were immediately blended in a Stomacher for 3 min, sufficient inoculum was added to provide ≈10^7 CFU/g *L. monocytogenes* and the samples remixed for another 2 min. The contents of the Stomacher bags were then aseptically transferred to sterile 250 mL polycarbonate bottles (Nalgene).

Incubation and sampling

Inoculated meat samples were stored at 37°C. Periodically 2-4 g samples were aseptically transferred to Stomacher bags. Samples were diluted with 0.1% peptone water (1:10 w/v), mixed and plated on tryptose soy agar (Difco) with a Spiral Plater (Model D, Spiral Systems Instruments, Cincinnati, OH). After incubation for 24–48 hr at 37°C, colonies were counted with either a Bacteria Colony Counter (Model 500A, Spiral Systems Instruments) or manually. Nitrite analyses were conducted on an identically processed but uninoculated set of samples by the AOAC (1990) procedure of section 973.31. Six samples were testing having pH 4.0 or 4.7 with 0, 150 or 300 μg/g nitrite.

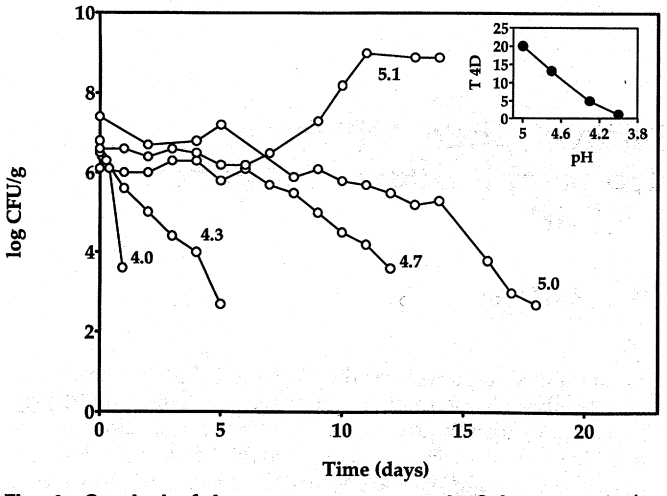

Fig. 1—Survival of *L. monocytogenes* at 37°C in meats (3% NaCl) acidified to various pH values and with 150 ppm added nitrite. Inset, effect of pH on T_{4D}.

Modeling

The complete design of 5 pH levels by three nitrite levels was replicated twice. Bacterial counts from samples with declining populations (non-growth) were fitted to a nonlinear inactivation model (Buchanan et al., 1994; Whiting, 1993) using the curve fitting program of Sigma Plot version 4.0 (Jandel Scientific, Corte Madera, CA). Values of lag time, initial decimal reduction time (D value), and time for four logs decline (T_{4D}) were determined (Whiting, 1993). Analyses of variance and polynomial regressions were performed with the RS/1 statistical program (BBN Software Products Corp., Cambridge, MA). Samples where growth occurred were modeled with the Gompertz equation (Buchanan and Phillips, 1990).

RESULTS & DISCUSSION

AT PH 5.1 AN EXTENDED PERIOD with a slow decline of 1 log or less in *L. monocytogenes* numbers was followed by growth. The inactivation model was fitted to the declining period and the Gompertz equation to the growth period. At that pH, the lag time was much longer and the growth rate slower than predicted by the growth model. Concurrently, the observed T_{4D} times during the initial period were much longer than those predicted by the inactivation model. At pH 5.0 and 4.7 the increases in numbers predicted by the growth model were not observed in the batters. The observed T_{4D} times were longer than expected although within the confidence range of the model. This may also reflect a slight amount of growth in an environment favoring overall decline. At pH ≤ 5.0, inactivation was observed with no growth. The survival data with 150 ppm nitrite showed that as pH decreased, inactivation rates increased (Fig. 1, Table 1). Lag, D and T_{4D} values were shorter at each lower pH ($p \leq 0.01$). T_{4D} values decreased from 22 to 11 days within the usual pH range of fermented meat products (pH 5.0 to 4.7). The regression equation to estimate the T_{4D} for this meat system, with the highest F and R^2 values was

$$T_{4D\ 37°C} = 68.99 - 46.81pH + 7.42pH^2 + 0.000012[NO_2]^2$$

where time is in days and nitrite in ppm ($R^2 = 0.95$ and $F = 120.1$). Evidently, from the equation, the nitrite levels had limited effects on estimated inactivation times. The amount of nitrite added to the batter had no significant effect upon the parameters by analysis of variance ($p > 0.05$).

The predicted T_{4D} values were calculated from the nonlinear-pH quadratic model of Buchanan et al. (1994). T_{4D} values from the model based upon lactic acid concentration instead of pH values were also compared (not shown) but agreement was not as good, probably because any added amount of acid would lower pH much further in the broth than in the meat. The model did not include 300 ppm nitrite within its range, therefore, these values were not calculated. Predictions of the inactivation model for 0 ppm nitrite batters were within the precision expected of a model which encompasses D values from a few hours to months. Lowering the pH below 5.0 greatly hastened the decline (as well as slowing growth) indicating that effective fermentation is important in ensuring control of *L. monocytogenes* in such uncooked products.

The approximately tenfold decrease in survival times predicted by the model with addition of 150 ppm nitrite was not observed. The nitrite analyses showed 56 ppm residual nitrite after 1 day in pH 4.0 and 4.7 samples and 28 and 84 ppm after 2 days, respectively. This generally agreed with the model for declining nitrite by Nordin (1969) and the measurements in thuringer sausage by Dethmers et al. (1975) which indicated the residual nitrite in this product would be expected to be < 20 ppm after 1 to 2 days. Junttila et al. (1989) concluded that nitrite and nitrate additions to a meat product at officially approved levels did not cause elimination of *L. monocytogenes*. The observed T_{4D} were closer to calculated T_{4D} with residual nitrite at pH 4.0. They were closer to no residual nitrite at pH 4.7 than to added nitrite.

Table 1—Predicted and observed survival of *Listeria monocytogenes* in an acidified meat batter with nitrite at 37°C and 3.2% brine

		Observed			Predicted T_{4D}[a]		Observed growth Gompertz equation		Predicted growth	
pH	Added $NaNO_2$ (ppm)	Lag (days)	D value (days)	T4D (days)	Value (days)	Lower, upper 95% CL (days)	Lag period (days)	Growth rate (log(CFU/mL)/day)	Lag period (days)	Growth rate (log(CFU/mL/day)
5.1	0	—[b]	12.4	53.2	12.5	3.9, 39.8	9.2	0.30	1.2	5.5
	150	—[b]	10.8	46.6	1.3	0.3, 5.7	8.4	0.24	4.1	5.2
	300	—[b]	8.6	37.2	[c]		15.6	0.73		
5.0	0	9.7	3.0	21.6	11.9	4.0, 35.1			1.6	4.5
	150	7.6	3.1	20.0	1.3	0.3, 4.9			5.9	4.5
	300	7.2	3.5	21.0						
4.7	0	6.1	1.3	11.4	9.6	4.0, 22.9			4.4	2.4
	150	5.8	1.8	13.2	1.0	0.3, 3.2			20.0	2.6
	300	6.6	2.1	15.0						
4.3	0	0.00	1.2	5.3	5.9	3.0, 11.3				
	150	0.10	1.1	4.9	0.7	0.3, 1.7				
	300	0.19	1.4	5.9						
4.0	0	0.01	0.10	0.5	3.3	1.9, 5.8				
	150	0.05	0.25	1.1	0.5	0.2, 1.0				
	300	0.02	0.19	0.8						

[a] T_{4D} time for 4 logs decline. Values calculated from the nonlinear-pH model for *Listeria* survival given in Buchanan et al. (1994).
[b] Decline before growth. Lag period not distinguishable from decline (D).
[c] Blank values in prediction columns represent conditions outside the ranges of the models.

The studies of *L. monocytogenes* survival during fermentation showed several logs decline after the fermentation period of 1 to 2 days, particularly when starter cultures were used (Glass and Doyle, 1989; Sabel et al., 1991, Farber et al., 1993). The modeling studies in broth (Buchanan et al., 1993, 1994) and meat (this study) showed longer survival times than reported in fermented products. In particular, a lag period before the decline began was observed in both types of model studies. There are several possible explanations for the apparent absence of *L. monocytogenes* after fermentation in meat products. Accurate quantitative detection of reduced populations ($<10^2$ CFU/g) from inoculations in raw product of 10^4 CFU/g is difficult. Acid injured cells are unable to grow on selective media used in product studies (Siragusa and Dickson, 1992). There is potential for production of bacteriocins by the lactic acid bacteria of the starter cultures (Berry et al., 1990; Nettles and Barefoot, 1993). Additional studies with these types of products are needed before the models can be relied upon for accurate predictions and their use should be limited to acquiring first estimates of the likely behavior of the pathogen.

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Ms received 12/14/93; revised 4/8/94; accepted 4/26/94.

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